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Influence of the host plants on the efficacy of hear NPV against *Helicoverpa armigera*

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Abstract
Biological control of a cosmopolitan pest *Helicoverpa armigera* through baculoviruses is emerging as safe and best viable alternative control measures over the chemical methods. But there use in the field is very limited, due to their short persistence, reduced viability and slow killing of pest. The virulence of the virus is significantly influenced by the host plant chemicals against the insect pest. The infectivity of the nuclear polyhedrosis virus, HearNPV to *H. armigera* was significantly reduced when viral occlusion bodies were exposed to the plant phenolic compounds. The degree of inactivation of HearNPV was greater on chickpea than that reported on pigeonpea, fieldbean, soybean, blackgram and greengram host plants.

Keywords: *Helicoverpa armigera*, Hearnpv, phenols, tannins and peroxidase

Introduction

Helicoverpa armigera, also known as cotton bollworm, corn earworm, or tomato fruit borer, is a highly polyphagous insect pest from the family Noctuidae and order Lepidoptera. Sarwar *et al.* (2014) reported pest attacks over 180 cultivated plant species including cereals, legumes, vegetables, fruits, and oilseeds such as chickpea, pigeonpea, tomato, okra, cotton, groundnut, and maize. Its life cycle includes four stages—egg, larva, pupa, and adult—and completes in about 30–34 days at 28°C. The larval stage is the most destructive, feeding on leaves and boring into pods to consume developing seeds, sometimes causing total crop failure. A single larva can damage 25–30 pods in its lifetime (Kumar *et al.*, 2019) [20]. Economic losses due to *H. armigera* in India are estimated at over ₹35,000 million annually. It causes 4.2–77% yield losses in chickpea and damages worth $350 million in pigeonpea and $2 billion across various semi-arid crops (Sharma *et al.*, 2005) [28]. This pest consumes nearly half of all pesticides used in India. However, chemical control affects natural enemies and poses environmental risks. Due to the drawbacks of chemical insecticides, biopesticides, particularly microbial agents like *Metarhizium rileyi*, *Beauveria bassiana*, *Bacillus thuringiensis*, and *H. armigera* nucleopolyhedrovirus (HearNPV), are preferred for eco-friendly pest management (Rabindra and Jayaraj, 1988 [25]; Pande *et al.*, 2005) [24].

HearNPV, a member of the Baculoviridae family, has been widely used as part of Integrated Pest Management (IPM). Baculoviruses are insect-specific, double-stranded DNA viruses that form protective polyhedral occlusion bodies (POBs), enabling persistence in the environment. Baculoviruses have a long history as effective and environment-friendly insect control agents in field crops, vegetables, forests and pastures (Moscardi 1999) [22], and the genetics of baculoviruses is well known (Herniou *et al.* 2003) [18]. Upon ingestion, the virus dissolves in the larval midgut, infects cells, replicates, and causes larval death through liquefaction. Baculoviruses have proven effective in managing lepidopteran pests without harming beneficial organisms, offering a sustainable alternative to chemical pesticides.

Biopesticides based on insect viruses, particularly baculoviruses, specificity, environmental safety, and effectiveness make them attractive, yet they represent only a small share of the global biopesticide market, which is dominated by chemical insecticides (Trimmer, 2017) [31]. In crops like chickpea and cotton, reduced virus efficacy is also attributed to plant surface phytochemicals (Rabindra *et al.*, 1994 [26]; Williams, 2017) [32]. An array of phytochemicals, including phenolics, alkaloids, nonprotein amino acids, and terpenoids may influence the susceptibility of insects to viral diseases (Felton *et al.*, 1987) [15]. Hence, this present study aims at studying the interaction of host plant chemicals that play a role in influencing the virulence of HearNPV.

Material and Methods

Maintaining of the host insects

Field collected *H. armigera* larvae were brought to the laboratory and examined for parasitoid association and microbial contamination through standard entomological and pathological screening. Healthy larvae were individually reared on a semi-synthetic diet as per Shorey and Hale (1965) [29] and allowed to pupate. The culture was maintained in an incubator at 25 °C, 70% relative humidity, with a 14:10 h light–dark photoperiod. Adults emerged within 17–20 days, and the eggs laid by these moths were used to maintain the test insect colony for subsequent laboratory experiments.

Collection and extraction of baculovirus

H. armigera larvae showing typical HearNPV symptoms were collected from the field and transported to NBAIR, Hebbal (Bengaluru). Each larva was homogenized for 4 min in 5 ml sterile distilled water with a chilled pestle and mortar to release occlusion bodies (OBs). The homogenate was passed through glass wool, which was rinsed with an additional 0.5 ml sterile water. The filtrate was centrifuged at 15,000 × g for 5 min; the supernatant was discarded, and the pellet was washed with 2 ml sterile water and recentrifuged under the same conditions. The final pellet was resuspended in 1 ml sterile distilled water and stored at 4 °C. OBs were enumerated with a Neubauer haemocytometer (depth 0.1 mm) under phase-contrast

microscopy, using 100–1000-fold dilutions following Evans and Shapiro (1997) [12]. The stock suspension was adjusted to 1×10^9 OBs ml^{-1} and kept at 4 °C until required.

Bioassay of *Hear*NPV under laboratory conditions

The diet surface contamination method (Srinivasa *et al.*, 2008) [30] was used to evaluate the efficacy of HearNPV against *Helicoverpa armigera* under laboratory conditions. A virus suspension with 1×10^5 OBs/ml was prepared, and eight concentrations (1×10^4 to 1×10^{10} OBs/ml) and control were tested. Ten microliters of virus suspension were applied to the diet surface using a sterile micropipette and spread evenly with a glass rod. Pre-starved second and early third instar larvae were placed individually into glass vials containing the treated artificial diet, with ten larvae per treatment and three replications. Control larvae received only distilled water. Vials were incubated at 25 ± 1°C, with larvae transferred to fresh diet as needed. Mortality was recorded daily from day 3 to day 10. NPV infection was confirmed by symptom observation and dark-field microscopy. Mortality data were analysed using probit analysis in SPSS to determine LC_{50} at 95% confidence. Larval mortality in control was corrected using Abbott's correction formula (Abbott, 1925) [1].

Bio-chemical analysis of host plant foliage

Studies were carried out to establish possible relationship between biochemical factors of host plant foliage and susceptibility of *Hear*NPV by quantifying the following biochemical factors using standard techniques.

Leaf surface pH

Matured leaves of different host plants *viz.*, pigeonpea, chickpea, field bean, soybean, greengram and blackgram were plucked randomly from the field and brought to the laboratory. Two fresh leaves were dipped separately in beakers containing 100 ml of distilled water and stirred well for 10-15 min by holding the petioles (Andrews and Sikorowski, 1973) [4]. Then the beaker containing water was placed in a digital pH meter and pH of the leaf surface was recorded.

Crude leaf extract pH

About 300 mg of frozen foliage of each host plant was taken for assessing the leaf extract pH. After removing the leaf midribs using scissors, the sample was homogenized in 10 ml chilled, deionized distilled water in a mini blender for 15 seconds at low speed as described by Appel and Maines (1995) [5]. This resulted in a leaf macerate with particle size similar to those observed in the midgut of *H. armigera* larvae. The leaf homogenate was transferred to a 20 ml scintillation vial and pH was determined using a digital pH meter.

Total phenol content

The total phenol content of leaves was estimated by adopting the standard technique (Malick and Singh, 1980) [21] with little modification.

Reagents

1. **Folin-Ciocalteau reagent (FCR):** commercial grade reagent was diluted in 1:1 ratio with distilled water. 2.5ml of FC reagent was diluted with 2.5ml of distilled water.
2. **7.5% Sodium carbonate solution:** 7.5g of sodium carbonate in 100ml of distilled water.
3. **Standard Gallic acid solution:** A stock gallic acid solution was prepared containing 1mg of gallic acid /1ml of water. 250mg of gallic acid was dissolved in 250ml of distilled water. This solution was diluted in the ratio 1:10 to obtain 100µg of gallic acid per ml of working standard solution.

Sample extraction

2g of fresh leaf sample was homogenised in 10ml of distilled water using pestle and mortar and the extract volume was made upto 100ml in volumetric flask The extract was centrifuged at 10000 rpm for 5 minutes.1ml of the supernatant was further used for the estimation of phenols.

Estimation of phenols

1ml of the sample aliquot was diluted with 1ml of distilled water and 1 ml of FCR was added and mixed thoroughly. Exactly after five minutes 5ml of 7.5% sodium carbonate solution was added and kept on boiling water bath at 30°C for 10 minutes. The tubes were covered and allowed to stand for 30 min at room temperature before the absorbance was read at 760 nm spectrometrically. The Folin-Ciocalteau reagent is sensitive to reducing compounds including polyphenols, thereby producing a blue colour upon reaction. This blue colour was measured spectrophotometrically. A standard graph was constructed with gallic acid in the range of 20-100µg. The total phenol content was expressed as mg per gram of fresh sample.

Total tannin content

The leaf tannin content was estimated by adopting the standard technique (Schanderi, 1970) [27].

Reagents

1. **Folin-Denis reagent:** 100g sodium tungstate and 20g phosphomolybdic acid were dissolved in 750ml of distilled water in a flask and then 50 ml of Phosphoric acid was added. The mixture was refluxed for 2 hours and volume was made to 1 litre.
2. **Sodium carbonate solution:** 350g of sodium carbonate was dissolved in water and made upto to 1000ml and filtered through glasswool after allowing it to stand overnight.
3. **Standard tannic acid solution:** Tannic acid stock solution was prepared by dissolving 100mg of tannic acid in 1000 ml of distilled water.
4. **Working standard solution:** 5ml of stock solution was diluted in 100 ml of distilled water. One ml contains 50 µg tannic acid.

Sample extraction

Hundred mg of oven-dried and powdered leaf sample was extracted by boiling in 80 ml of distilled water for 30 minutes and cooled. The extract was then centrifuged at 2000 rpm for 20 minutes. The supernatant was used for the estimation of total tannins (Schanderi, 1970) [27].

Estimation of tannins

One ml of the aliquot was transferred to a volumetric flask containing 75 ml of distilled water. To this, 5 ml of Folin-Denis reagent, 10 ml of sodium carbonate solution were added and volume made up to 100 ml with distilled water. After incubation for 30 minutes, solution was stirred well and absorbance was read at 700nm against a reagent blank using spectrophotometer. A standard graph was constructed with tannic acid in the range of 0-100µg as a standard. The total tannin content was expressed as mg per gram of oven-dried sample.

Total peroxidase activity

The total peroxidase enzyme activity of leaves was estimated by adopting the standard technique (Chance and Maehly, 1952) [7].

Reagents

1. **Hydrogen peroxide solution:** 0.14 ml of 30 per cent hydrogen peroxide was diluted to 100ml with 0.1M sodium phosphate buffer, pH 7.0 to get 12.3mM solution.

2. **Guaiacol solution (20 mM):** One ml of 1M guaiacol solution was diluted to 100ml to get 20mM solution. It was stored frozen until use.

Preparation of enzyme extract

Hundred mg of acetone powder was extracted with 10ml of ice-cold 0.1M sodium phosphate buffer, pH 7.0 containing 0.1 per cent polyvinylpyrrolidone in a pestle and mortar for 2-5 minutes. The slurry was centrifuged at 10,000 rpm for 20 minutes at 4°C. The supernatant was used as enzyme source for the assay of total peroxidase (Chance and Maehly, 1952) [7].

Assay of peroxidase activity

Three ml of 0.1M sodium phosphate buffer (pH 7.0), 0.1 ml of 20 mM guaiacol solution and 0.1 ml of 12.3mM hydrogen peroxide were taken in a cuvette. The reaction was initiated by the addition of 0.1ml of enzyme extract pre-incubated at 25°C and the increase in absorbance was measured at a time interval of 30 seconds upto 5 minutes at 470 nm in a spectrophotometer. The activity of the enzyme was calculated from the linear part of the progress curve. One unit of peroxidase was defined as increase in absorbance of 0.01/min. under the standard assay conditions and the activity was expressed as units/g acetone powder.

Midgut pH of host larvae

On hatching, 25 neonate larvae of *H. armigera* were transferred to the respective host plants *viz*., chickpea, pigeonpea, field bean, soybean, greengram and blackgram reared up to late fifth instar stage. Then, fully fed larvae of uniform size numbering ten were selected from different host plants and were immobilized on ice. Larval dissection was carried out. First, without damaging the internal parts, larvae were cut open along the mid-dorsal line. Middle portion of gut was lifted using sterile forceps and gently pulled out by separating the trachea adhering to the midgut. The midgut part was cut off and immediately stored in ice cold 2 ml scintillation vial. The midgut was macerated using sterilized glass rod and mixed well to release the inner contents. Then the midgut pH was recorded using a standard digital pH meter. The midgut pH of the given sample was expressed as average pH of ten larvae.

Statistical Analysis

The data which are obtained from all the experiments were subjected to the statistical analysis to evaluate effects of treatments. Analysis was carried out by completely randomized design (CRD) using software WASP-2 tool (Duncan, 1995) [11].

Results and Discussion

Efficacy of HearNPV NBAIR V on the second and third instar larva of *H. armigera*

At 3 DAT larval mortality rate ranged from 10 to 46.66 per cent in 2nd instar larva. While in 3rd instar the larval mortality ranged from 3.33 to 36.66 per cent at 1 $x10^4$ OB/ml and 1 $x10^{10}$ OB/ml respectively. In the second instar larva the treatment 1 x 10^4 OB/ml after 4 DAT showed 13.33 per cent larval mortality and at 1 $x10^{10}$ OB/ml at 4 DAT showed 60 per cent

Table 1: Bio-efficacy of HearNPV NBAIR V isolate against different larval instars of *H. armigera*

Days after treatment	Larval Mortality (%) at various concentrations														control
	1X10^4		1X10^5		1X10^6		1X10^7		1X10^8		1X10^9		1X10^{10}		
	2nd Instar	3rd Instar	2nd Instar	3rd Instar	2nd Instar	3rd Instar	2nd Instar	3rd Instar	2nd Instar	3rd Instar	2nd Instar	3rd Instar	2nd Instar	3rd Instar	
3 DAT	0.00	3.33	16.67	10.00	26.67	13.33	30.00	16.67	36.67	20.00	43.33	26.67	46.67	36.67	
	(2.825)^{d}	(1.552)^{de}	(4.099)^{cd}	(2.825)^{cd}	(5.191)^{bc}	(3.669)^{bc}	(5.523)a^{b}	(4.099)^{bc}	(6.084)^{ab}	(4.43)^{abc}	(6.611)^{ab}	(5.191)^{ab}	(6.859)^{a}	(6.084)^{a}	(0.707)^{e}
4 DAT	13.33	6.67	20.00	16.67	53.33	26.67	33.33	26.67	43.33	36.67	50.00	46.67	60.00	56.67	
	(3.669)^{d}	(2.396)^{e}	(4.528)^{d}	(4.099)^{d}	(7.33)^{ab}	(5.191)^{cd}	(5.803)^{c}	(5.191)^{cd}	(6.611)^{bc}	(6.084)^{bc}	(7.083)^{ab}	(6.859)^{ab}	(7.76)^{a}	(7.554)^{a}	(0.707)^{e}
5 DAT	16.67	20.00	20.00	56.67	60.00	60.00	46.67	56.67	70.00	63.33	76.67	70.00	80.00	66.67	
	(4.099)^{d}	(4.528 ^{c}	(4.528)^{d}	(7.554)^{b}	(7.76)^{b}	(7.76)^{ab}	(6.859)^{c}	(7.536)^{b}	(8.382)^{ab}	(7.984)^{ab}	(8.78)^{a}	(8.396)^{a}	(8.961)^{a}	(8.19) ^{ab}	(0.709)^{d}
6 DAT	20.00	23.33	23.33	53.33	66.67	56.67	56.67	63.33	73.33	60.00	80.00	73.33	83.33	76.67	
	(4.528)^{d}	(4.859)^{d}	(4.859)^{d}	(7.307)^{c}	(8.19)^{bc}	(7.554)^{c}	(7.554)^{c}	(7.984)^{abc}	(8.574)^{ab}	(7.778)^{bc}	(8.972)^{a}	(8.588)^{ab}	(9.153)^{a}	(8.769)^{a}	(0.707)^{e}
7 DAT	23.33	26.67	36.67	56.67	76.67	60.00	66.67	66.67	73.33	70.00	80.00	76.67	86.67	80.00	
	(4.859)^{e}	(5.191)^{e}	(6.084)^{d}	(7.554)^{d}	(8.78)^{abc}	(7.76)^{cd}	(8.19)^{c}	(8.19)^{bcd}	(8.588)^{bc}	(8.382)^{abc}	(8.961)^{ab}	(8.78)^{ab}	(9.333)^{a}	(8.972)^{a}	(0.707)^{e}
8 DAT	26.67	30.00	30.00	53.33	80.00	63.33	70.00	70.00	76.67	73.33	83.33	76.67	90.00	83.33	
	(5.191)^{d}	(5.523)^{d}	(5.471)^{d}	(7.307)^{c}	(8.972)^{abc}	(7.966)^{bc}	(8.396)^{c}	(8.382)^{ab}	(8.78)^{bc}	(8.574)^{ab}	(9.153)a^{b}	(8.78)^{ab}	(9.513)^{a}	(9.153)^{a}	(0.707)^{e}
9 DAT	43.33	33.33	53.33	53.33	93.33	63.33	73.33	73.33	76.67	76.67	83.33	80.00	90.00	83.33	
	(6.611)f	(5.803)^{d}	(7.33)^{e}	(7.083)^{c}	(9.684)^{a}	(7.984)^{b}	(8.588)^{d}	(8.588)^{ab}	(8.78)^{cd}	(8.78)^{a}	(9.153)^{ab}	(8.972)^{a}	(9.513)^{ab}	(8.972)^{a}	(0.707)^{e}

Note: Values are mean of three replications. Figures in parentheses are √x+1 transformed value. Mean values with different superscript within the same column represent a

Significant difference as determined by DMRT ($p \leq 0.05$)

Larval mortality. The larval mortality rate of 3rd instar larva in the same observation ranged from 6.66 per cent to 56.66 per cent. At 5 DAT larval mortality ranged from 16 to 80 per cent and 20 to 60.66 per cent in the second and third instar larva respectively.

The larval mortality rate was 20 to 83.33 per cent in the second instar larva and 23.33 to 76.66 percent in third instar larva on exposure at 6 DAT. The per cent larval mortality ranged from 23.33 to 86.66 and 26.66 to 80 per cent at second and third instar larva respectively on 7 DAT. The larval mortality rate was 26.66 and 43.33 per cent respectively on 8 DAT and 9 DAT at the concentration of 1 x 10^4 OB/ml. The 90% larval mortality was observed on 8th and 9th day percent at the concentration of 1 x 10^{10} OB/ml in the second instar larva. Similarly, the larval mortality rate is 30 and 33.33 per cent on 8 DAT and 9 DAT at 1 x 10^4 OB/ml. The larval mortality rate was 83.33 percent on both 8th and 9th day in 3rd instar larva (Table 1). The minimum of 50 per cent larval mortality occurred at the 5th and 6th day as compared to the 8 DAT and 9 DAT showing this is a potential isolate to carry into the field against the *H. armigera.* At lower concentration the maximum larval mortality of larva was observed in both the larval instars indicating concentration is lethal to the *H. armigera* that the cost to be incurred on the entomopathogen can be reduced. So that in field conditions cost of cultivation can also be minimized.

Nawaz *et al.* (2019) [23] reported that mean percent larval mortality comparison showed that maximum larval mortality (95.00 ± 2.05%) was seen at 1 × 10^{10} OB which was 9.95 times higher than control, followed by 95.00 ± 1.99% at 1 × 10^9 OB which was 9.95 times higher than control.

Plate 1: Bioassay of HearNPV against second instar larvae of *H. armigera*

Table 2: pH of host plant leaf surface, leaf extracts and larval midgut

Treatments	Leaf surface	Crude leaf extract	Midgut pH
T_1: Chickpea	5.68 (2.486) [f]	5.89 (2.528) [e]	6.11 (2.528) [e]
T_2: Pigeonpea	6.38 (2.624) [d]	6.13 (2.574) [d]	6.45 (2.574) [d]
T3: Field bean	6.18 (2.585) [e]	6.18 (2.585) [d]	6.34 (2.585) [d]
T4: Green gram	6.47 (2.641) [c]	6.88 (2.716) [c]	6.96 (2.716) [c]
T5: Black gram	6.86 (2.714) [b]	7.09 (2.756) [b]	7.19 (2.756) [b]
T6: Soybean	7.27 (2.789) [a]	7.50 (2.828) [a]	7.56 (2.828) [a]
T7: Semisynthetic diet	0.00 (0.707) [g]	0.00 (0.707) [f]	0.00 (0.707) [f]

Note: Values are mean of five replications. Figures in parentheses are √x+1 transformed value. Mean values with different superscript within the same column represent a significant difference as determined by DMRT ($p \leq 0.05$)

Leaf surface pH

The leaf surface pH differed significantly among the host plants studied. Leaf surface pH ranged from 5.68 (chickpea) to 7.27 (soybean). Leaf surface pH was slightly alkaline (7.27) in soybean, acidic in field bean (6.18) and pigeonpea (6.38) and near neutral (6.86 and 6.47) in blackgram and greengram respectively, extremely acidic in chickpea (5.68) (Table 2).

Crude leaf extract pH

Significant differences were observed in the pH of crude leaf extracts of different host plants. Like the leaf surface pH, the pH of blackgram and soybean leaf extract was nearly alkaline (6.12 and 7.56) whereas the leaf extracts of chickpea is (6.18) and pigeonpea (6.13) were acidic in nature. Chickpea extracts recorded extremely acidic pH (5.89). In general, the pH values of leaf extracts of all the host plants were slightly lower than that of leaf surface (Table 2).

Midgut pH of host larva

The midgut pH values of third instar *H. armigera* larva varied with the host plants they consumed. The midgut contents were slightly alkaline to alkaline, ranging from 7.56 to 8.40. The midgut pH values differed significantly with *H. armigera* larva reared on semi synthetic diet (Table 2).

The less larval mortality on chickpea was observed which may be attributed to the low pH of acidic range 5.68 responsible for the inactivation of baculovirus this is just hypothetical assumption based on the present investigations. Similar findings were also reported. The presence of organic acids on chickpea leaves causes the surface to have a very low leaf pH, in the range 0.4-1.3, similar to the pH of the solutions tested here. Concentrations equivalent to those found in chickpea leaf extracts had a pH of 1.4; this is well within the ranges reported as inactivating NPV OB (Ignoffo and Garcia, 1966 [19]; Gudaukas and Canerday, 1968) thus the acidity of the leaf surfaces may explain the potent inactivation. The most significant feature that should be in a formulation is long-term stability (Airenne *et al.*, 2013) [2]. One of the factors, the pH value, significantly affects the shelf life of the baculovirus formulations. The pH of the solution prior to the addition of NPV should be 5–7. Because OBs structures dissolve in alkaline substrates, it causes virulence to decrease in a short time (Batista *et al.*, 2001) [6].

Table 3: Total phenols, tannins and peroxidase activity in leaves of different host plants

Sl. No.	Treatments	Phenols		Tannins		Peroxidase Activity	
		OD@ 765nm	Total Phenols (mg/g)	OD@ 720nm	Tannin conten (mg/g)	OD@ 240 nm	Total peroxidase activity (mM^{-1} min^{-1} g^{-1})
1	T1: Chickpea	1.513[c]	5.527[c]	0.463[d]	44.89[d]	1.833[b]	15.72[bc]
2	T2: Pigeonpea	1.860[a]	6.807[a]	1.953[a]	186.07[a]	1.973[a]	4.97[d]
3	T3: Field bean	1.733b	6.353[b]	1.650b	155.35[b]	13.43[d]	14.70[bc]
4	T4: Soybean	1.927[a]	6.563[ab]	1.860[a]	176.95[a]	1.973[a]	16.38[b]
5	T5: Black gram	0.890[d]	3.173[d]	1.577[b]	150.74[b]	1.243[e]	32.96[a]
6	T6: Green gram	1.757[b]	6.327[b]	1.390[c]	133.68[c]	1.663[c]	13.46[c]

Note: Values are mean of five replications. Mean values with different superscript within the same column represent a significant difference as determined by DMRT ($p \leq 0.05$)

Total phenol content

The total phenol content varied significantly among different host plants (Table 3). The phenol content was highest in the pigeonpea leaves (6.807mg/g) followed by fieldbean and soybean (6.353 and 6.563 mg/g, respectively). The other two host plants tested i.e. chickpea and blackgram had much lower levels of total phenols (5.527 and 3.173 mg/g, respectively). This is more consistent with the hypothesis that involves a physical change to the OB that persists even when the phytochemicals have been removed after exposure. Felton and Duffey (1990) [13] proposed that the binding of plant phenolic to OB could lead to impaired solubilisation and release of virions. Other mechanisms of inactivation have included chemical binding leading to aggregation of OB impairing virion release, masking through phenolic binding or direct damage (Cory and Hoover, 2006) [8]. Since leaves have the highest density of glandular hairs, the exudates of which inactivate the HaNPV, there is a higher degree of inactivation. Yearian and Young (1974) [33] also reported that polyhedra placed on the calyx, bract or bloom of cotton and the under surface of cotton leaves were protected more than those placed on the upper surface of either mature or terminal leaves.

Total tannin content

The results of total tannin content (mg/g of sample) in different host plants revealed that it was highest in the leaves of pigeonpea (186.07 mg/g of leaf) and soybean (176.95 mg/g of leaf) followed by field bean, blackgram, which recorded 155.35 and 150.74 mg/g of leaf, and the lowest tannin was observed in chickpea (44.89 mg/g of leaf) (Table 3). Felton *et al.* (1987) [15]. The predominant orthodihydroxyphenols, rutin and chlorogenic acid, from the tomato plant, *Lycopersicon esculentum*, inhibited the infectivity of NPV in larval *Heliothis zea* and in cell culture of *Trichoplusia ni*.

Total peroxidase activity

The total peroxidase activity in the fresh leaves of different host plants during crop growth period differed significantly. The blackgram recorded significantly higher peroxidase activity (32.96 units/g) followed by soybean, chickpea field bean and greengram (16.38, 15.72, 13.45 of acetone powder, respectively) and the least was found in pigeonpea 4.97 units/g acetone powder) (Table 3).The above results clearly indicated that pigeonpea, field bean, soybean, greengram had the highest levels of total phenols, tannins and the peroxidase activity followed by that of chickpea. The leaves had much lower levels of total phenols, tannins and peroxidase activity.

Aminu *et al.* (2023) [3] reported that exposure to 2 organic compounds produced naturally at high concentrations from chickpea leaf trichome exudates dramatically reduced HearNPV infectivity, while trichomes of soybeans were reported to have no significant effect on the increased activity of AfMNPV against *Trichoplusia ni* (Hay *et al.* 2020) [17].

Foliar peroxidase activity also was correlated positively with the production of free radicals, including highly damaging reactive oxygen species (ROS) such as H2O2 and OH; these radical species were strongly linked to inhibition of baculoviral disease because inhibition was markedly reversed in the presence of scavengers of these species.

The inhibitory influence of phenolics on NPV to account for the effect of plant oxidative enzymes. Besides high constitutive levels of rutin and chlorogenic acid, tomato foliage contains high levels of polyphenol oxidase (PPO) and peroxidase (POD) activities (Felton *et al.*, 1989 [14]; Duffey, 1986 [9, 10]; Duffey and Felton, 1989) [14]. When foliage is physically damaged, such as by chewing of insect larvae, these oxidases rapidly convert chlorogenic acid to the o-quinone, chlorogenoquinone, Moreover, this oxidation continues during the digestion of the food in the midgut. Quinone formation in tomato foliage reduces the growth rate of noctuid herbivores (Felton *et al.*, 1989) [14] but the effectiveness of NPV as a mortality factor may be compromised in plant systems with significant levels of PPO and phenols. In addition to reducing the incidence of viral infection, antibiotic levels of PPO and phenolics may have far greater impact by reducing the number of infective OBs released in the environment.

Conclusion

Further research is essential to understand how these chemical interactions affect herbivore populations and their natural enemies. The antiviral properties of phytochemicals could significantly influence the compatibility of baculoviruses with host plants that express high levels of such defensive compounds. Uncovering the mechanisms by which phytochemicals inhibit baculoviral infections will enable the development of more effective formulations that can overcome or reduce these inhibitory effects.

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